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Agilent Multimode Inlet

Large Volume Injection Tutorial

Agilent Technologies

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Multimode Inlet Tutorial

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Overview

A growing number of researchers are exploring large volume injection (LVI) techniques to improve existing analyses. With traditional injection approaches in capillary gas chromatography, most inlets and columns can only handle 1-2 µL at a time. Attempts to increase the injection volume lead to broadened and distorted analyte peaks, large and long solvent peak tails, and saturated or damaged detectors.

The desire to increase the injection volume is normally to improve trace analysis. By introducing more of the sample to the system, the mass of analyte reaching the detector will be proportionately increased, resulting in larger peak areas and peak heights. If the baseline noise is kept constant, larger peak heights mean greater signal to noise ratios and lower system detection limits. An additional benefit of LVI is the ability to reduce the amount of sample originally processed. For example, suppose a water sample contains 1000 ng/L of pollutant. If the current method extracts the pollutant and reconstitutes it in 1 mL of solvent, the concentration of analyte in the extract is 1000 ng/mL. A 1 µL injection of this extract puts 1 ng onto the column. Now assume that LVI allows a 10 µL injection volume. The researcher could start with 100 mL of sample, extract it with less solvent, and reconstitute it in 1 mL. A 10 µL injection puts 1 ng onto the column as before, but starts with an order of magnitude less sample (and likely, an order of magnitude less extraction solvent). Another advantage of using LVI is the decrease in solvent that actually reaches the detector. Usually, only 10-30% of the injection solvent actually enters the column and makes it to the detector.

LVI can be applied to injection volumes ranging from a few microliters up to 1 mL or more. In most LVI approaches, the sample solvent is selectively evaporated and removed from the inlet system before the analytes are transferred to the separation column. In this way, LVI is similar to nitrogen evaporation or rotary evaporation of the solvent, with the added benefit

of being performed in the GC inlet rather than in a fume hood. Analytes that would be lost during nitrogen evaporation may be retained in the inlet and successfully analyzed via LVI. Furthermore, the LVI process can be automated and is reproducible. As in the other evaporation techniques, the LVI approach is a function of the solvent type, the inlet temperature, the vent flow of evaporation gas, and the analyte boiling point. In addition, the inlet pressure during evaporation and the inlet liner have an impact on the rate of solvent removal and analyte recovery. These parameters will be described in more detail in the tutorial.

Hot Splitless

For most researchers considering LVI, their current methods are using hot splitless injection. This proven and reliable sample introduction method has worked well for almost 40 years; however, it does present some challenges to the sample integrity and to the method developer. First, the inlet must be hot enough to flash vaporize the solvent and analytes so that the resulting vapor cloud can be transferred to the column. The inlet liner volume must be sufficiently large to contain this vapor cloud. If the liner volume is too small, the vaporized sample can exit the liner and reach reactive surfaces, leading to analyte loss. In addition, the pressure wave generated by the vaporized sample can push back against the incoming carrier gas and enter sensitive pressure and flow control systems. Using the Agilent pressure/flow calculator, a 1 µL injection of acetone into an inlet at 240 °C and 14.5 psig expands to 288 µL of gas. Most inlet liners for standard split/splitless inlets have a nominal volume of 1 mL. An increase of injection volume to only 3.5 µL under these conditions creates a vapor cloud of 1 mL which could easily overflow the inlet liner.

Hot splitless injection also creates a challenging environment for thermally unstable analytes. Compounds such as the organochlorine pesticides DDT and endrin can rearrange to form breakdown compounds. This process is accelerated with the inlet

temperatures normally used to analyze them. Effective chemical deactivation of the liner can minimize analyte breakdown. However, high inlet temperatures can decrease the lifetime of deactivated liners.

Another challenge created by hot splitless injection is the opportunity for needle fractionation or analyte discrimination. The needle temperature increases as the sample is being transferred from the syringe to the inlet because the needle is in contact with the septum. The rise in needle temperature can cause the solvent to "boil" away and deposit high boiling analytes inside the needle. To avoid this fractionation problem, some researchers load a solvent plug into the syringe first and then draw up the desired sample volume. The thought is that the solvent plug will wash any deposits into the inlet. An effective way to address this problem is to make a high speed injection. This minimizes the time the needle is in contact with the septum and the time the sample touches the needle. Even with these issues, hot splitless injection can be made to work well. An alternative approach, such as cold splitless can address these concerns and improve the analysis results.

Cold Splitless

The Agilent Multimode Inlet (MMI) uses the same liners and consumables as a standard split/splitless inlet, making it compatible with existing hot split and splitless methods. However, its temperature programmability allows it to perform cold split and splitless analyses as well. In cold splitless mode, the MMI is cooled to a temperature below the normal boiling point of the sample solvent so that when the sample is injected, no vaporization takes place. The injection is simply a liquid transfer from the syringe to the inlet. Once the syringe is removed from the inlet, the inlet is heated to vaporize the sample and transfer it to the column. The solvent vaporizes first and moves to column, allowing analyte focusing to take place as in normal hot splitless injections. The analytes subsequently vaporize and move to the column. The main advantage is that the analytes vaporize at the

lowest possible inlet temperature, rather than at a constant high temperature, minimizing thermal degradation while still allowing a wide range of analytes to vaporize. Cold splitless operations also do not thermally stress the liner as harshly as hot splitless does, prolonging its usable life. Cold splitless can also extend the amount of sample that can be injected in some cases. If a slow inlet temperature program is used, the solvent can be vaporized slowly and not overflow the liner volume. As long as the analytes can be refocused on the column, slow inlet temperature programs cause no detrimental effects to the chromatography.

Solvent Vent

The solvent vent mode is how the MMI is able to do LVI. In solvent vent mode, the inlet is kept at a low initial temperature during sample application. Pneumatically, the inlet is in split mode with a low inlet pressure. The flow of gas through the inlet liner and out to vent removes the evaporating solvent. The sample is injected so that the incoming liquid is deposited on the liner wall and the solvent evaporates at a similar rate. Once the entire sample has been injected, the inlet switches to a splitless mode for analyte transfer. The inlet is then heated to vaporize the concentrated sample and any remaining solvent and they are transferred to the column. After a sufficient period to ensure the sample transfer, the inlet is then switched to a purge mode to allow any remaining material in the inlet liner to be removed to waste. During the sample injection and solvent venting period, the GC oven has been held at an appropriate temperature to allow the solvent to refocus the analytes on the column. When this refocusing is complete, the oven is then programmed to perform the separation.

Tutorial

You can choose to use a current hot splitless method to follow this tutorial or the checkout sample that came with your instrument. The tutorial will use the Flame Ionization Detector (FID) MDL checkout sample (p/n 5188-5372) to demonstrate the method development process. This sample contains four hydrocarbons (C13, C14, C15, and C16) in isooctane. Flame ionization detection is used as this will show you more of the LVI behavior for analytes that elute closely to the solvent and the solvent itself.

Step 1 - Hot Splitless

In order to calibrate your system for recovery calculations, you will need to run your current method. For your first step, simply run your sample by your existing hot splitless method or use the conditions below for the FID MDL alkane mix.

Column and sample

Type	HP-5, 30 m x 0.32 mm x 0.25 µm (19091J-413)
Sample	FID MDL Checkout (5188-5372)
Column flow	4 mL/min
Column mode	Constant flow

MMI

Mode	Splitless
Inlet temperature	250 °C
Initial time	5 min
Rate 1	0 °C/min
Purge time	2 min
Purge flow	60 mL/min
Septum purge	3 mL/min

FID

Temperature	300 °C
H2 flow	30 mL/min
Air flow	400 mL/min
Makeup flow (N2)	25 mL/min
Lit offset	Typically 2 pA

Oven

Initial temperature	50 °C
Initial time	2 min
Rate 1	20 °C/min
Final temperature	200 °C
Final time	0 min

ALS

Sample washes	2
Sample pumps	6
Injection volume	1 µL
Syringe size	10 µL
PreInj Solvent A washes	3
PreInj Solvent B washes	3
PostInj Solvent A washes	3
PostInj Solvent B washes	3
Viscosity delay	0
Plunger speed	Fast
PreInjection dwell	0
PostInjection dwell	0

*Wash solvent — Isooctane

Data system

Data rate	20 Hz

You may want to run the sample 2-3 times to get an average for the peak areas. Figure 1 shows the typical results for the FID MDL sample under these conditions.

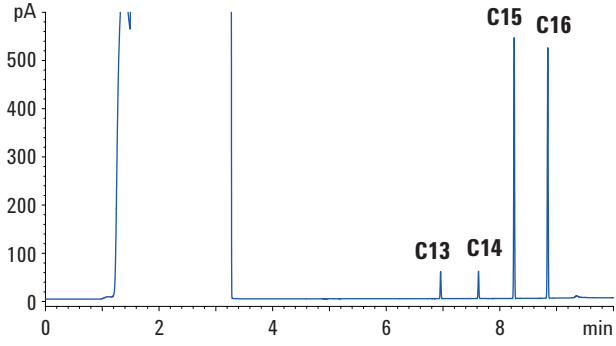

Figure 1 Typical hot splitless FID MDL sample results

Step 2 - Cold Splitless

To make a cold splitless analysis, you will need to change the inlet temperature. Set the inlet initial temperature to 5-10 °C below the normal boiling point of your sample solvent. Hold this temperature for 0.1 minutes, then program the inlet at 720 °C/min up to the inlet temperature for the hot splitless method. See the conditions below for the FID MDL method (only the MMI conditions are given, the rest are all the same as for hot splitless).

MMI

Mode	Splitless
Inlet temperature	90 °C
Initial time	0.1 min
Rate 1	720 °C
Final temperature	250 °C
Final time	5 min
Purge time	2 min
Purge flow	60 mL/min
Septum purge	3 mL/min

Compare the peak areas, peak widths, and peak shapes for the hot and cold splitless modes. Figure 2 shows the typical cold splitless results for the FID MDL sample. For this sample, the results are almost identical between hot and cold splitless.

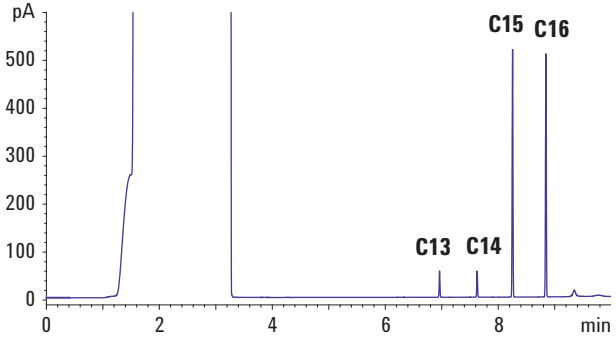

Figure 2 Typical cold splitless FID MDL sample results

Step 3 - Solvent Vent

Now change the MMI mode to PTV Solvent Vent. Notice that the **Solvent Elimination Calculator** button appears (Figure 3).

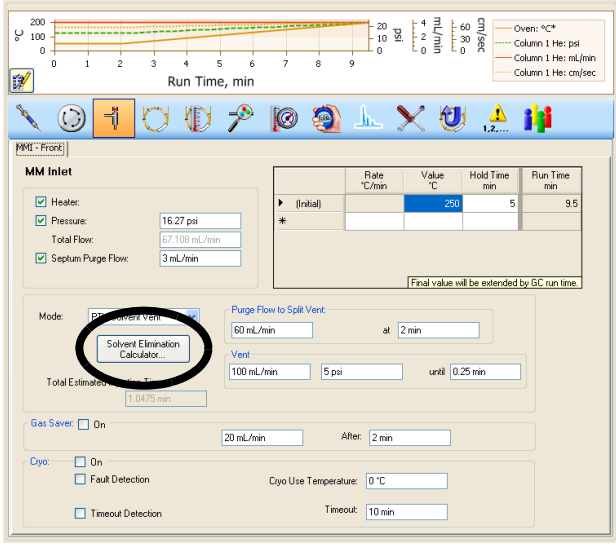

Figure 3 Accessing the Solvent Elimination Calculator

This calculator was designed to help you determine reasonable starting conditions for your LVI method. Click the **Solvent Elimination Calculator** button to start the calculator. In the first screen (Figure 4 on page 13), you are asked for several pieces of information. You should be able to provide the sample solvent and your desired injection volume. The third piece of information is the boiling point of the earliest eluting analyte. If you know this, select the temperature closest to the value; otherwise, you can leave it at 150 °C as this will help retain a wide range of analytes. For the FID MDL

sample, set the solvent to isooctane, the injection volume to 5 µL, and the boiling point to 200 °C. Click **Next** to go to the calculation screen.

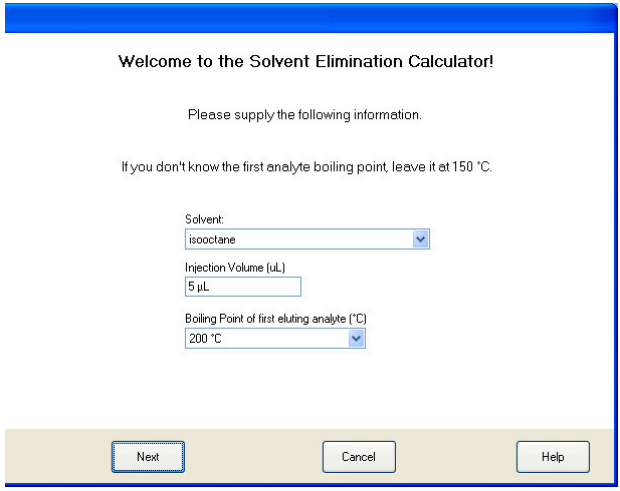

Figure 4 Solvent Elimination Calculator

Figure 5 on page 14 shows the calculation screen. Taking the information that you provided, the calculator has used an initial set of instrument conditions to determine the solvent elimination rate according to fundamental theory. This “Elimination Rate” does not account for other factors specific to LVI and is normally too fast as determined from practical experience. The “Suggested Injection Rate” does consider these factors and is designed to leave a small amount of solvent in the liner at the end of the venting period. This solvent serves as a liquid “trap” for the more volatile analytes and promotes their recovery. The “Suggested Vent Time” is determined by dividing the injection volume by the “Suggested Injection Rate”.

NOTE **For more information, see the LVI Method Help in your instrument control software.**

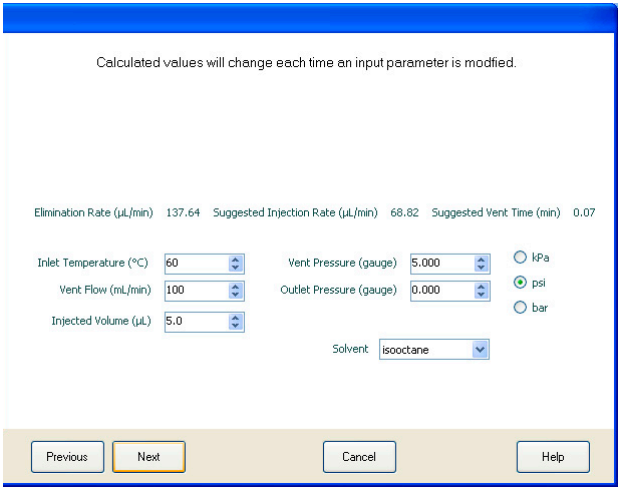

Figure 5 Solvent Elimination Calculator variables

The variables for determining elimination rate are user-settable in the lower portion of the window. To illustrate how these parameters interact, try the following changes (marked in **black**) and record the Elimination Rate value in Table 1.

Table 1 Elimination Rate worksheet

Inlet temp (°C)	Vent Flow (mL/min)	Inject Vol (µL)	Vent Press (psig)	Outlet Press (psig)	Solvent	Elimination Rate (µL/min)
60	100	5	5	0	Isooctane	137.64
40	100	5	5	0	Isooctane	
60	**50**	5	5	0	Isooctane	
60	100	5	**2**	0	Isooctane	
60	100	5	5	**2**	Isooctane	
60	100	5	5	0	**Hexane**	

Note that a small change in inlet temperature has a significant impact on elimination rate. Vent flow has a linear effect such that a decrease by a factor of two in vent flow gives an equal decrease in elimination rate. As the vent pressure decreases, the elimination rate increases. Bear in mind that the vent pressure also impacts how much solvent reaches the column during venting. As the vent pressure is increased, more solvent is loaded onto the column before the analytes are transferred. Finally, the solvent type, specifically its normal boiling point, has a substantial impact on the elimination rate.

To continue with the tutorial, change the calculator values back to those shown in Figure 5 on page 14 and listed below. Click **Next** to move to the method changes screen (Figure 6 on page 16). The calculator "knows" the syringe that is currently installed and will only allow 50% of that volume to be injected. If you ask for more, the calculator will warn you that the system cannot make the injection and give you a choice as to how to proceed.

MMI

Mode	Solvent Vent
Inlet temperature	60 °C
Initial time	0.07 min
Rate 1	720 °C
Final temperature	250 °C
Final time	5 min
Vent flow	100 mL/min
Vent pressure	5 psig
Vent time	0.07 min

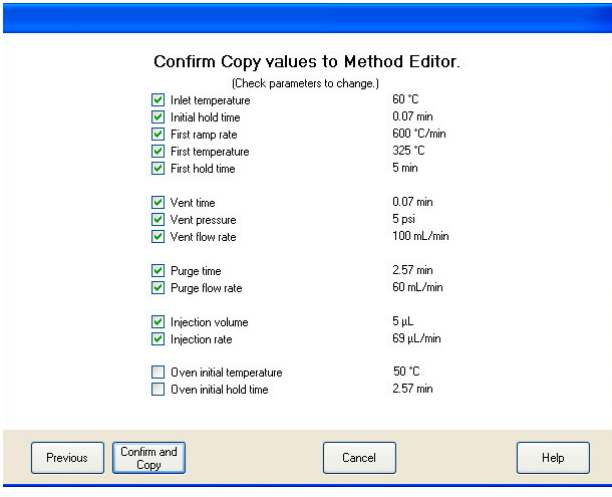
Confirm Copy values to Method Editor.
(Check parameters to change.)
Inlet temperature 60 °C
Initial hold time 0.07 min
First ramp rate 600 °C/min
First temperature 325 °C
First hold time 5 min
Vent time 0.07 min
Vent pressure 5 psi
Vent flow rate 100 mL/min
Purge time 2.57 min
Purge flow rate 60 mL/min
Injection volume 5 µL
Injection rate 69 µL/min
Oven initial temperature 50 °C
Oven initial hold time 2.57 min
Previous
Confirm and Copy
Cancel
Help

Figure 6 Method changes to download to the Method Editor (Solvent Elimination Calculator)

This screen shows you all of the method changes that will be downloaded to the Edit Parameters screen. You can choose to accept or reject any of these parameters.

The Oven initial temperature and hold times are not automatically checked in case your method requires these values to be unchanged (e.g. you have a Retention Time Locked method). For the FID MDL sample, click **Confirm and Copy** and then **Ok** in the Edit Parameters screen.

Run the analysis and compare the peak areas between this run and your original hot splitless analysis. Figure 7 shows an overlay of these two runs. The dotted trace is the original hot splitless injection result and the solid trace is the solvent vent result.

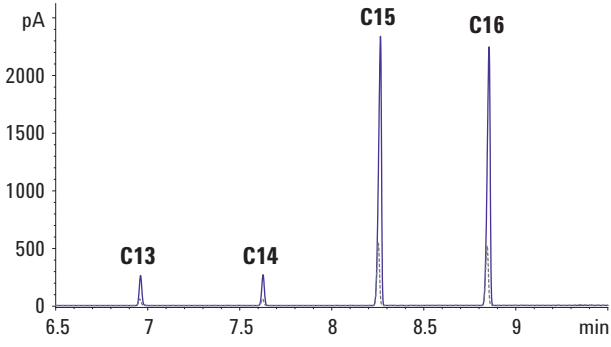

Figure 7 Overlay of the original hot splitless injection result (dotted line) and the solvent vent result (solid line)

Table 2 on page 18 compares the resulting peak areas for the two runs. The peak widths for the analytes are essentially the same for both runs. The result is that the peaks are five times taller and show an increase of five fold in signal to noise ratio.

Table 2 Resulting peak areas for hot splitless and solvent vent runs

Inlet mode	Solvent area	C13 area	C14 area	C15 area	C16 area
1 µL Hot splitless	17113114	56	56	555	554
5 µL Solvent vent	36859256	261	268	2622	2596
Solvent vent recovery	44%	93%	96%	94%	94%

In Table 2, solvent vent recovery was calculated by dividing the solvent vent run areas by five times the hot splitless areas. For the analytes, the recoveries are almost 100% and are almost identical, indicating that the solvent vent conditions gave essentially a five-fold improvement in instrument detection limits. Notice that the solvent recovery is only 44%. This means that from the 5 µL sample injection, only 2.2 µL entered the column.

Let's extend this to larger injection volumes. Install a larger syringe into the autosampler such as a 25 or 50 µL. Increase the injection volume to 10 µL and use the Solvent Elimination Calculator to determine initial conditions. For the FID MDL sample, the MMI conditions are now:

MMI

Mode	Solvent Vent
Inlet temperature	60 °C
Initial time	0.15 min
Rate 1	720 °C
Final temperature	250 °C
Final time	5 min
Vent flow	100 mL/min
Vent pressure	5 psig
Vent time	0.15 min

Figure 8 shows a close-up of the analyte peaks with a 10 µL injection. The first two peaks are still reasonably symmetrical but the last two peaks clearly show fronting. This is due partially to column overload and to the amount of solvent transferred to the column.

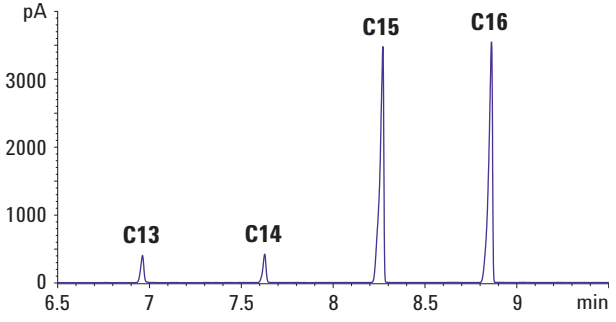

Figure 8 Analyte peaks using a 10 µL injection

Table 3 shows the recovery from the initial hot splitless run. Notice that the 10 µL Solvent Vent recoveries are slightly lower than the 5 µL Solvent Vent run shown in Table 3. This is confirmed by the lower solvent recovery. To improve this, the inlet temperature could be lowered while keeping all other parameters the same or by shortening the vent time slightly. In both cases, more solvent would be left behind to help trap the C13. Of these two approaches, the inlet temperature has a larger effect on trapping the early eluting analytes.

Table 3 **Recovery from the initial hot splitless run**

Inlet mode	Solvent area	C13 area	C14 area	C15 area	C16 area
1 µL Hot splitless	17113114	56	56	555	554
10 µL Solvent vent	59579040	493	510	5106	5208
Solvent vent recovery	35%	88%	91%	92%	94%

To scale up to larger injection volumes, the easiest way is to increase the vent time proportionally. You can use the Solvent Elimination Calculator to explore this relationship. For a 50 µL injection, a vent time of 0.75 minutes is needed. The injection parameters for the FID MDL sample are given below. In order to avoid overloading the column, the FID MDL sample was diluted 1:10 in isooctane.

MMI

Mode	Solvent Vent
Inlet temperature	60 °C
Initial time	0.75 min
Rate 1	720 °C
Final temperature	250 °C
Final time	5 min
Vent flow	100 mL/min
Vent pressure	5 psig
Vent time	0.75 min

The resulting chromatogram is shown in Figure 9 on page 21. The peak shapes are obviously distorted, a result of too much solvent being transferred to the column. You can fix such a problem in several ways. Simply increasing the vent time even more will reduce the amount of solvent in the column. Figure 10 on page 22 shows the resulting chromatogram with a vent time of 0.90 minutes instead of 0.75 minutes. The peak shapes are greatly improved and are very similar to the 5 µL chromatogram shown in Figure 7 on page 17. Other approaches to reduce the solvent being transferred to the column are to increase the vent flow, decrease the vent pressure, or increase the inlet temperature during the vent period. The Solvent Elimination Calculator can show you how much a change in the parameters will affect the elimination rate. Two other approaches can also help improve analyte recovery and peak shape. Using a retention gap will aid in refocusing the analyte

peaks and improve peak shape. A second method is to include some retaining material in the liner such as glass wool or packing. Material in the liner aids in holding the analytes during solvent venting and allows more solvent to be vented. When you use retaining material in the liner, you need to be aware of losing analytes due to irreversible adsorption.

For more information and sample applications, please refer to the Agilent Web site (http://www.chem.agilent.com/en-US/Products/Instruments/gc/multimodeinlet/pages/default.aspx).

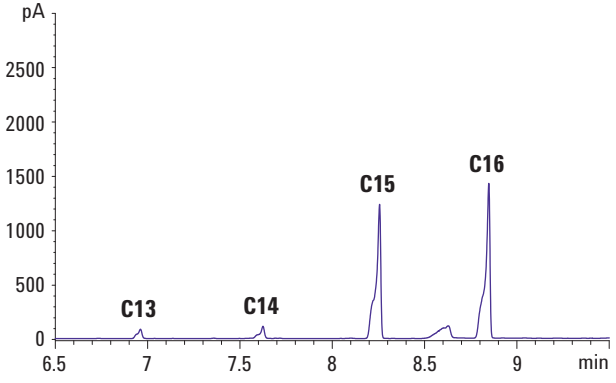

Figure 9 50 µL injection of FID MDL sample diluted 1:10 in isooctane

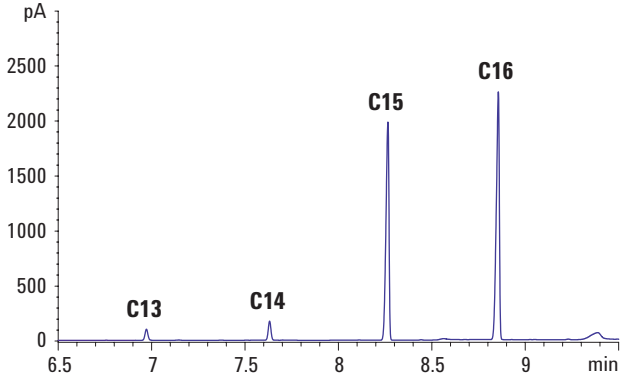

Figure 10 50 µL injection resulting chromatogram with a vent time of 0.90 minutes instead of 0.75 minutes

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